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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/593,316	06/13/2000	John Clark	730/002	5627

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EXAMINER	
LI, QIAN JANICE	

ART UNIT	PAPER NUMBER
1632	

DATE MAILED: 01/30/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/593,316	CLARK ET AL.	
	Examiner	Art Unit	
	Q. Janice Li	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 September 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-7,13-17,22 and 27-37* is/are pending in the application.

4a) Of the above claim(s) *7,17,22 and 27-32* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6,13-16 and 33-37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *13 June 2000* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a)☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1)☒ Notice of References Cited (PTO-892)
2)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3)☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4)☐ Interview Summary (PTO-413) Paper No(s). _____ .
5)☐ Notice of Informal Patent Application (PTO-152)
6)☐ Other: .

DETAILED ACTION

In view of the Appeal Brief filed on 9/803, PROSECUTION IS HEREBY REOPENED. New grounds of rejections are set forth below.

To avoid abandonment of the application, appellant must exercise one of the following two options:

(1) file a reply under 37 CFR 1.111 (if this Office action is non-final) or a reply under 37 CFR 1.113 (if this Office action is final); or,

(2) request reinstatement of the appeal.

If reinstatement of the appeal is requested, such request must be accompanied by a supplemental appeal brief, but no new amendments, affidavits (37 CFR 1.130, 1.131 or 1.132) or other evidence are permitted. See 37 CFR 1.193(b)(2).

Please note that claims 13-16 will be rejoined for examination in this Office action. The arguments presented in the Appeal Brief would be addressed to the extent that they apply to the current rejection.

Claims 1-6, 13-16, and 33-37 are under current examination.

Claim Rejections - 35 USC § 101 & 112

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-6 and 33-37 are rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a credible asserted or a well-established utility.

The claims are drawn to ovine tissue and cells devoid of antibody-detectable Galα(1,3) Gal determinants, ovine cells homozygous for inactivation of an α(1,3)GT gene, and an ovine animal homozygous for inactivation of an α(1,3)GT gene. The specification asserts that the tissue and cells could be used for xenotransplantation.

However, the specification fails to disclose a live-born lamb heterozygous for α1,3GT inactivation. Without production of a live lamb, breeding is not reduced since a fetus could not be used for breeding a viable lamb homozygous for α1,3GT inactivation. Accordingly, there is no credible utility for claimed ovine and ovine cells.

With respect to cells having *homozygous* inactivation of α1,3GT gene, the specification contemplates they could also be obtained by knocking out both alleles of α1,3GT gene by homologous recombination. However, this approach has been proven to be extremely difficult to achieve in the art (See discussion under 35 USC § 112, 1st paragraph), and has yet to be on record for any species of farm animals. Since the claimed homozygous ovine cells have not been provided by the specification, the asserted utility is not considered credible.

With respect to cells having *heterozygous* inactivation of α1,3GT gene, the Galα1,3Gal epitope is autosomal dominant, thus the ovine fetuses or ovine cells

heterozygous for inactivation of α1,3GT gene have no phenotype, i.e. they are not devoid of antibody-detectable Galα1,3Gal determinants. Thus, they do not have a specific and substantial utility. Applicant is referred to the Revised Utility Examination Guidelines published December 21, 1999 in the Federal Register, Volume 64, Number 244, pages 71441-71442 for the required *specific* and *substantial* utility. "A CLAIMED INVENTION MUST HAVE A SPECIFIC AND SUBSTANTIAL UTILITY. THIS REQUIREMENT EXCLUDES 'THROW-AWAY' 'UNSUBSTANTIAL', OR 'NONSPECIFIC' UTILITIES," (column 3, 3rd paragraph of page 71441).

Further, neither the specification as filed nor any art of record discloses or suggests any specific property or activity for the ovine animals, which would give the animals and their cells a well-established utility.

Accordingly, the claimed invention is not supported by a substantial asserted utility, a creditable asserted utility, or a well-established utility for the reasons set forth above.

Claims 1-6 and 33-37 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. Specifically, since the claimed invention is not supported by either a credible asserted or a well established utility for the reasons set forth above, one skilled in the art would not know how to use the claimed invention so that it would operate as intended without undue experimentation.

WRITTEN DESCRIPTION

Upon further consideration, previous written description rejection is withdrawn.

ENABLEMENT REQUIREMENT

Claims 1-6, 13-16, and 33-37 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The factors to be considered when determining whether the disclosure satisfies the enablement requirements and whether undue experimentation would be required to make and use the claimed invention are summarized in *In re Wands*, (858 F2d 731, 737, 8 USPQ 2d 1400, 1404, (Fed Cir.1988)). These factors include but are not limited to the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, the breadth of the claims, and amount of direction provided. The factors most relevant to this rejection are the nature of the claims relative to the state of the art and the levels of the skilled in the art, and whether sufficient amount of direction or guidance are provided in the specification to enable one of skill in the art to practice the claimed invention.

Claim 1 recites "Ovine tissue devoid of antibody-detectable Gal α (1,3)Gal determinants", claim 3 recites "isolated ovine cell or tissue that expresses glycosyl transferase enzymes but does not detectably express α(1,3)GT", claim 4 recites "an ovine cell which is heterologous or homozygous for inactivation of an α(1,3)GT gene",

claim 6 recites "an ovine animal that is homozygous for inactivation of an α1,3GT gene". Dependent claims 33-37 specify various types of cells inactivated for an α1,3GT gene. However, the claimed cells and animals homozygous for inactivation of an α(1,3)GT gene, tissue and organs devoid of Gal α (1,3)Gal determinants have not been reduced to practice at the time the application was filed. Therefore, further consideration of cloning methods provided by the specification and the state of the art at the time of the effective filing date is *necessary*.

Claim 13 is directed to producing an ovine that is homozygous for inactivation of an α1,3GT gene comprising providing an ovine embryo of cells heterologous or homologous for inactivation of an α1,3GT gene, engrafting the embryo into the uterus, birth an ovine, and further mating the ovine if the birthed ovine has only one inactivated allele of α1,3GT gene.

As an initial matter, no cell homologous for inactivation of an α1,3GT gene has been provided by the specification, nor an embryonic stem cell lacking one allele of an α1,3GT gene could have been provided by the specification, because ES cells are not available for sheep at the time of the effective filing date. With respect to using somatic cells for gene targeting, a post-filing art published by the applicants (*Denning et al*, Nat Biotech 2001 June;19:559) teaches the difficulties of somatic cell targeting, "A SUBSTANTIAL NUMBER OF COLONIES WITH ONLY TARGETED CELLS SENESCED BEFORE THEY COULD BE PREPARED FOR NUCLEAR TRANSFER. THE HIGH ATTRITION RATE OF TARGETED CLONAL POPULATIONS SUITABLE FOR NUCLEAR TRANSFER REPRESENTS ONE OF THE MAJOR HURDLES OF GENE TARGETING IN PRIMARY SOMATIC CELLS" (left column, page 560).

In the specification, the claimed method is illustrated by using a genetic construct inactivating one allele of α1,3GT gene in a fibroblast cell, and generating embryos by nuclear transfer of the targeted donor fibroblast cells. The specification contemplates producing sheep heterozygous for inactivated α1,3GT using nuclear transfer, then mating the heterozygous knockouts (Specification, page 41, lines 14-17). However, at the time of the effective filing date, a live-born lamb comprising the *heterologous* inactivation of α1,3GT gene has not been reduced to practice. Thus, the heterologous disrupted lamb, the starting material for mating purposes, is lacking, one can not mate a heterozygous fetus to obtain a sheep devoid of Gal α (1,3)Gal determinants.

In view of the state of the art, the skilled artisans teach that fetuses not surviving to term often reflect the real difficulty and challenge in animal cloning. *Yanagimachi* (Mol Cell Endocrinol 2002;187:241-8) teaches, at a post-filing date, that "CLONING EFFICIENCY-AS DETERMINED BY THE PROPORTION OF LIVE OFFSPRING DEVELOPED FROM ALL OOCYTES THAT RECEIVED DONOR CELL NUCLEI-IS LOW REGARDLESS OF THE CELL TYPE (INCLUDING, EMBRYONIC STEM CELLS) AND ANIMAL SPECIES USED. IN ALL ANIMALS EXCEPT OF JAPANESE BLACK BEEF CATTLE, THE VAST MAJORITY OF CLONED EMBRYOS PERISH BEFORE REACHING FULL TERM" (Abstract), and "THUS FAR, CLONED OFFSPRING THAT SURVIVED BIRTH AND REACHED ADULTHOOD WERE THE EXCEPTION RATHER THAN THE RULE (page 243, left column, emphasis added). This teaching again is contrary to applicant's assertion that once the sequences and construct are available, producing the animal is just routine experimentation. *Yanagimachi* goes on to teach, "THIS LOW EFFICIENCY OF CLONING SEEMS TO BE DUE LARGELY TO FAULTY EPIGENETIC REPROGRAMMING OF DONOR CELL NUCLEI AFTER TRANSFER INTO RECIPIENT OOCYTES. CLONED

EMBRYOS WITH MAJOR EPIGENETIC ERRORS DIE BEFORE OR SOON AFTER IMPLANTATION" (abstract). *Wells et al* (Trends Biotechnol 2003:21:428-32) teach that the continuous loss of clones throughout pregnancy and high mortality during the perinatal period raise serious animal welfare concerns and these losses can mostly be attributed to faulty epigenetic reprogramming of the donor cell genome, resulting in major dysregulation of gene expression (paragraph bridging left & right column in page 1). Thus, in light of the state of the art and the levels of the skilled, lack of survival to term in applicants' ovine fetuses having heterozygous inactivation of an α1,3GT gene is less likely an accident, but reflects problems in epigenetic reprogramming and major genetic dysregulation. MPEP teaches, "WHEN CONSIDERING THE FACTORS RELATING TO A DETERMINATION OF NON-ENABLEMENT, IF ALL THE OTHER FACTORS POINT TOWARD ENABLEMENT, THEN THE ABSENCE OF WORKING EXAMPLES WILL NOT BY ITSELF RENDER THE INVENTION NON-ENABLED." "LACK OF A WORKING EXAMPLE, HOWEVER, IS A FACTOR TO BE CONSIDERED, ESPECIALLY IN A CASE INVOLVING AN UNPREDICTABLE AND UNDEVELOPED ART." (MPEP 2164.02, 03) The court states, "IF INDIVIDUALS OF SKILL IN THE ART STATE THAT A PARTICULAR INVENTION IS NOT POSSIBLE YEARS AFTER THE FILING DATE, THAT WOULD BE EVIDENCE THAT THE DISCLOSED INVENTION WAS NOT POSSIBLE AT THE TIME OF FILING AND SHOULD BE CONSIDERED. IN *IN RE WRIGHT*, 999 F.2D 1557, 1562, 27 USPQ2D 1510, 1513-14 (FED. CIR. 1993). Accordingly, taking as a whole the state of the art, the levels of the skilled in the art, the disclosure of the specification, and the lack of working examples for the homozygous inactivation of both ovine cells and ovine animals, it is reasonable to raise a doubt on the enablement of instant claims.

Claims 14 and 15 are directed to obtaining ovine cells and tissues from an ovine animal having disruption of both alleles of an α1,3GT gene, the method is not enabled

because as indicated foregoing, the starting material (i.e. the ovine animal), thus the basis for harvesting the cells and organs is lacking.

The specification then contemplates, alternatively, cells from a heterozygous knockout can be targeted with an inactivation vector to inactivate α1,3GT gene on the other haplotype to generate homozygous knockout cells and embryo (Specification, page 41, lines 17-20). After final Office action, applicants submitted a recent publication as supporting evidence that such method could be used for producing homozygous knock out cells. A closer look at the submitted publication would find that the submitted publication (*Phelps et al*, Science 2003 Jan;299:411-4) does not support the instantly claimed method. *Phelps et al* (who describe piglets homozygous for α1,3GT inactivation), teach, "ALTHOUGH OUR INTENT WAS TO KNOCK OUT THE SECOND ALLELE OF THE α1,3GT GENE BY HOMOLOGOUS RECOMBINATION, THIS DID NOT OCCUR" (lines 1-4, right column, page 413, emphasis added), this teaching addresses the predictability of double knock-out in a cell. *Phelps et al* employed a toxin A selection method for selecting α1,3GT-negative cells and taught its importance in achieving the double knockout of α1,3GT gene. *Phelps et al* teach, "THE FACT THAT ONE NORMAL-SIZED ALLELE WAS OBSERVED (INSTEAD OF TWO SHORTER KNOCKOUT ALLELES) INDICATED THAT KNOCKOUT OF THE SECOND α1,3GT ALLELE WAS DUE TO MECHANISMS OTHER THAN TARGETED HOMOLOGOUS RECOMBINATION-MEDIATED DISRUPTION, PROMOTER DYSFUNCTION, OR MRNA MISSPLICING AND INSTABILITY" (mid- and right column, page 412), and "BECAUSE WE USED THIS POWERFUL SELECTION METHOD, WHICH ALLOWS US TO ISOLATE ANY EVENT THAT RESULTS IN LOSS OF α1,3GT ACTIVITY, WE DISCOVERED A MUTATION IN THE SECOND ALLELE OF THE α1,3GT GENE. HAD WE USED STANDARD SELECTION

METHODS WITH PUROMYCIN OR HYGROMYCIN, WE WOULD NOT HAVE FOUND THE MUTATION" " (right column, page 413, emphasis added). The specification fails to teach the selection methods taught by *Phelps et al*, thus, the method of Phelps et al does not support the enablement of the claimed invention at the time the application was filed. The statue requires, "THE SPECIFICATION SHALL CONTAIN A WRITTEN DESCRIPTION OF THE INVENTION, AND OF THE MANNER AND PROCESS OF MAKING AND USING IT, IN SUCH FULL, CLEAR, CONCISE, AND EXACT TERMS AS TO ENABLE ANY PERSON SKILLED IN THE ART TO WHICH IT PERTAINS, OR WITH WHICH IT IS MOST NEARLY CONNECTED, TO MAKE AND USE THE SAME AND SHALL SET FORTH THE BEST MODE CONTEMPLATED BY THE INVENTOR OF CARRYING OUT HIS INVENTION". Accordingly, the Phelps reference does not provide enablement for making of a homozygous α1,3GT knockout sheep. The specification as filed fails to teach or produce a homozygous knockout cell in the way *Phelps et al* did, and the specification fails to generate a heterozygous knockout sheep that could be used for breeding, thus, the homozygous knockout sheep could not have been produced. Therefore, the basis for the assertion is flawed.

Further, it should be noted if fertilized eggs are used in the claimed process, they won't divide sufficient number of times to allow the selection process (30 to 45 times of division are required for selection).

While, the intent for citing the numerous references contradicting the applicants assertion is not to say that cloning by nuclear transfer to generate an ovine having homozygous inactivation of the α1,3GT gene can never be achieved, the intent is to provide art taught reasoning as to why the instant claims are not enabled at the time of the filing, and to illustrate the general state of the art in cloning, particularly nuclear

transfer cloning to properly determine whether additional and specific guidance should be provided by the specification.

Claim 16 is drawn to a method of xenotransplantation using ovine tissue devoid of antibody detectable Gal(1,3)Gal determinants, in other words, using tissue generated from ovine having homozygous knockout of the α1,3GT gene. As discussed foregoing, the specification fails to provide sufficient guidance or reduced to practice to provide the claimed ovine homozygous for α1,3GT gene inactivation, thus, the starting material for the claimed method is lacking.

Moreover, even if such ovine animal could be produced, the tissue or organ devoid of antibody detectable Gal(1,3)Gal determinants alone is insufficient for use in xenotransplantation. This is because Gal(1,3)Gal determinants (responsible for hyperacute rejection) are only one of the many factors that trigger xenograft responses. *Platt et al* (Nat Biotech 2002 Mar;20(3)231-2) clearly teach, "UNFORTUNATELY, SOLVING THE PROBLEM OF HYPERACUTE REJECTION DOES NOT MAKE XENOTRANSPLANTATION FEASIBLE, BUT RATHER REVEALS A MORE VEXING PROBLEM CALLED ACUTE VASCULAR REJECTION. ACUTE VASCULAR REJECTION, LIKE HYPERACUTE REJECTION, IS TRIGGERED BY ANTI-DONOR ANTIBODIES; HOWEVER, IN CONTRAST TO HYPERACUTE REJECTION, THESE ANTIBODIES ARE NOT DIRECTED EXCLUSIVELY AGAINST α1,3GAL, AND THE INVOLVEMENT OF THE COMPLEMENT SYSTEM IS FAR MORE SUBTLE" (Emphasis added). In view of such teaching, the claimed method does not appear to be enabled in the absence of evidence to the contrary.

Therefore, it is evident that at the time of the invention, the animal cloning practitioner, while acknowledging the significant potential of nuclear transfer cloning, still recognized that such practice was neither routine nor accepted, and awaited significant

development and guidance for its practice. Therefore, it is incumbent upon applicants to provide sufficient and enabling teachings within the specification to support the full scope of the claims. Here, the general knowledge and levels of skill in the art do not supplement the omitted disclosure, because specific, not general guidance is what is needed. Although the instant specification provides sheep cells having heterozygous inactivation of α1,3GT gene and fetuses that died in *uterus*, it is not enabled for the full scope because the specification fails to provide sufficient and specific guidance for the skilled artisan to reliably and reproducibly (routinely) produce what is now claimed. In summary, the teachings and guidance present in the specification, as a whole, represent an initial investigation into the feasibility of the development of a useful means for producing ovine animals devoid of α1,3Gal determinants, which awaits further development to practical levels.

Accordingly, in view of the limited guidance, the lack of predictability of the art and the nature and breadth of the claims, it would have required undue experimentation for one skilled in the art to make and/or use the claimed invention.

Response to Arguments

In the Appeal Brief, applicants assert "Sheep cells having an inactivated α1,3GT allele can readily be produced" (page 7, heading 1).

In response, the assertion is true for making somatic ovine cells with one allele being inactivated, but it is untrue for making so in ovine embryonic stem cells (ES cells), since ES cells are not yet available for ovine. Moreover, the homozygous knockout of

α1,3GT gene in the sheep does not appear to be a routine experimentation for either somatic cells or an ovine animal. This could be seen in applicant's disclosure and post-filing publication. In the specification, different genetic constructs were used for targeting fibroblast cells of different sheep species. Only one allele of α1,3GT gene was successfully inactivated in fibroblast cells of FD sheep with a construct targeting exon 4 of α1,3GT gene. Similar attempts failed to target cells of black Welsh mountain sheep or using a construct targeting exon 8 of α1,3GT gene (examples 4-6). Using nuclear transfer technology, ten recipients were determined to be pregnant, however, the specification fails to disclose the fate of the embryos and fetuses (example 6). In a post-filing publication submitted as IDS (*Denning et al,* Nat Biotech 2001 June;19:559), applicants disclose that 35 pregnancies were produced, the oldest fetuses died in *utero* at 118 and 130 days (term 148 days), and no live birth was obtained (page 560, right column). Apparently, the claimed cells, tissue and organs, and animals comprising a disruption of both alleles of an α1,3GT gene have not been reduced to practice as of the effective filing date, which indicated that sheep cells homozygous for inactivation of an α1,3GT gene have not been reduced by routine experimentation.

In the Appeal Brief, Applicants listed three ways of making ovine cells and animals homozygous for α1,3GT inactivation (page 9, heading 2). A detailed analysis of the means in the immediate preceding section has led to the conclusion that the teachings of the specification are insufficient to support the full scope of the claims.

With respect to the selection process for targeted donor fibroblast cells, the specification teaches selection for the homozygous knockout using a drug resistance

gene such as neo, similarly, two constructs with two different drug resistance genes could also be used for selection (Specification, page 40, line 24 to page 41, line 2). This is summarized in the Appeal Brief as a). a step-wise increase in antibiotic concentration to knockout both alleles, and referring to U.S. P. 5,589,369; and b). Two different antibiotics to sequentially knockout each allele (page 9). Applicants also assert that an antibody selection method is the equivalent to the toxin A method used by *Phelps et al.*

In reply, with respect to the teaching of US 5,589,369, the cited patent is not a cloning patent and it is directed to genetic modification of *mouse embryonic stem cells.* The ES cells for ovine has not been established, thus the starting material is lacking to apply the method taught by '369 patent on the sheep. Moreover, it is well known in the art that ES cells have significantly greater efficiency in cloning and gene targeting (*Wells et al*, TIBTEC 2003;21:428-32, particularly the conclusion), so when the sheep ES cells become available in the future, the obvious differences of mouse and human ES cells and resulting difficulties in cloning is likely to apply to farm animals. *Donovan and Gearhart* (Nat 2001 Nov;414:92-97) teach "HUMAN STEM CELL POPULATIONS PROLIFERATE MORE SLOWLY THAN THEIR MURINE COUNTERPARTS, DIFFERENTIATE MORE READILY AND THEIR CLONING EFFICIENCY IS VERY LOW" (last paragraph on page 95), "ONLY TIME WILL TELL WHETHER THE RESULTS OF CELL TRANSPLANTATION IN ANIMAL MODELS CAN BE RECAPITULATED IN HUMANS AND WHETHER IT WILL PROVE IMPOSSIBLE TO MAKE CERTAIN CELL TYPES FROM PLURIPOTENT STEM CELLS" (last paragraph on page 96). *Simerly et al* (Science 2003;300:297) report the molecular obstacles in cloning primates, and concludes, "PRIMATE NUCLEAR TRANSFER APPEARS TO BE CHALLENGED BY STRICTER MOLECULAR REQUIREMENTS FOR MITOTIC SPINDLE ASSEMBLY THAN IN OTHER MAMMALS", AND "WITH CURRENT

APPROACHES, NT TO PRODUCE EMBRYONIC STEM CELLS IN NONHUMAN PRIMATES MAY PROVE DIFFICULT—AND REPRODUCTIVE CLONING UNACHIEVABLE" (emphasis added).

With respect to the antibiotics selection method, whether it is the one step or two-step selection, the methods are considered "standard selection method" as referred to by *Phelps et al*, and *Phelps et al* clearly teach that such standard selection method would not reveal the mutation in the second allele or identify the possible presence of cells having homozygous inactivation of the α1,3GT gene.

With respect to the antibody selection method, the specification teaches, "*homozygous knockout cells can be made by targeting the other allele in the donor cells using a knockout vector, and selecting cells deficient in the particular surface antigen*". In the Appeal Brief, Applicant's counsel asserted that the antibody selection method is equivalent to toxin A selection, the method used by *Phelps et al*. However, the *Phelps* method is selecting for a gene recombination event, whereas the antibody selection is determining on a protein expression event, thus, the two could not be the equivalent.

In the Appeal Brief, Applicants further asserted that since homozygous knockout of an α1,3GT gene has been reduced to practice in mouse and pigs, sheep that are homozygous for inactivated α1,3GT can readily be produced. Applicants states that the central issue appears to the fact that the specification does not contain a report of a homozygous α1,3GT knockout sheep actually having been made, and Applicants asserted that the specification provides the sheep α1,3GT sequence, α1,3GT targeting vectors, α1,3GT knockout cells, and heterozygous α1,3GT knockout fetal sheep, thus,

the only elements missing from the examples can be achieved as a matter of routine experimentation (issues, page 5, and Arguments, page 6).

In response, as an initial matter, applicants are reminded that the cited mouse was produced with ES cells, and such cells are not available in ovine. Second, it is highly unpredictable from the production of a mouse to that of a large animal. *Hammer et al* (J Anim Sci 1986;63:269-78) report the production of transgenic mice, sheep and pigs; only transgenic mice exhibited an increase in growth due to the expression for the gene encoding human growth hormone (pages 276-277, Subsection: Effect of Foreign GH on Growth). *Mullins et al.*(J Clin Invest 1996 Apr;97:1557-60) state "A GIVEN CONSTRUCT MAY REACT VERY DIFFERENTLY FROM ONE SPECIES TO ANOTHER" (page 1559, Summary). *Wall et al* (J Dairy Sci 1997;80:2213-24) further report that "TRANSGENE EXPRESSION AND THE PHYSIOLOGICAL CONSEQUENCES OF TRANSGENE PRODUCTS IN LIVESTOCK ARE NOT ALWAYS PREDICTED IN TRANSGENIC MOUSE STUDIES" (page 2215, first paragraph). Third, because the genetic make-up and variations among different species of large animals, it is unpredictable from the results of a pig to that of a sheep, particularly in light of the comparison between the *Denning* reference (the attempt for making a heterozygous knock-out sheep and no live birth was obtained) and the *Dai* and *Lai* references (produced piglets knock-out of one allele of the pig α1,3GT gene). Without evidence to the contrary, transgene expression as well as knockouts phenotype in different species of genetically engineered animals is not consistent and varies according to the particular host's genetic background. Numerous pre- and post-filing arts indicated the inefficiency of producing genetically modified farm animals using nuclear transfer, even though this

is the most efficient approach for somatic cell cloning so far. For example, *Machaty et al* (Cloning Stem Cells 2002;4:21-27) teach with respect to making transgenic swine, "THE EFFICIENCY OF NUCLEAR TRANSFER IS NOT HIGH IN ANY SPECIES, BUT IN THE PIG IT IS ESPECIALLY LOW" (2nd paragraph, page 24). To this end, applicants submitted post-filing publications showing that heterologous and homozygous knockout of α1,3GT gene has been achieved in pig, yet, applicants' experience has shown that the efficiency of nuclear transfer is even lower in sheep compared to the pig because contrary to the pig, the heterozygous knockout of α1,3GT gene kills the ovine fetuses. Applicants are reminded the differences of genetic background in different animal species, and their influence on genetic manipulation. *Nebert et al* (Biochemical Pharmacol 1997 Feb;53:249-54) teach the "neighborhood effect" in genome modification between different mouse strains, "IT HAS BECOME INCREASINGLY APPRECIATED THAT (A) JUST WHERE A TRANSGENE IS INSERTED, (B) HOW MUCH OF THE GENE SEGMENT IS REMOVED, AND (C) HETEROGENEITY OF THE GENETIC BACKGROUND OF THE KNOCKOUT LINE CAN ALL CONTRIBUTE TO DRAMATICALLY DIFFERENT PHENOTYPES. *Linder* (Lab Animal 2001 May;30:34-9) teaches "THE GENETIC BACKGROUND AND THE SURROUNDING ENVIRONMENT ARE OFTEN OVERLOOKED PARAMETERS THAT CAN SIGNIFICANTLY AFFECT THE OBSERVED PHENOTYPE", "OTHER FACTORS INCLUDE MUTATIONS THAT ARE ACTUALLY HYPOMORPHS (I.E. MUTATIONS THAT CAUSE ONLY A PARTIAL DECREASE IN GENE EXPRESSION) RATHER THAN NULL ALLELES; COMPENSATORY PATHWAYS; AND TRANSGENESIS-SPECIFIC FACTORS, INCLUDING SITE OF INTEGRATION, TRANSGENE COPY NUMBER, AND INSERTIONAL MUTATIONS", "GENETIC BACKGROUND IS DEFINED AS A COLLECTION OF ALL GENES PRESENT IN AN ORGANISM THAT INFLUENCE A TRAIT OR TRAITS. WHILE MOST OF THE COMMONLY USED INBRED STRAINS SHARE A FAIRLY COMMON ORIGIN, EACH STRAIN HAS ITS OWN UNIQUE SET OF

CHARACTERISTICS OR BACKGROUND LESIONS", "THE PHENOTYPE OF MICE CARRYING A MODIFIED GENE WILL VARY DEPENDING ON THE GENETIC BACKGROUND BECAUSE OF THE PRESENCE OF GENETIC MODIFIERS (ALLELIC VARIANTS AT LOCI OTHER THAN THE ONE BEING GENETICALLY MODIFIED) IN THE INBRED STRAIN GENOME" (see entire article). The principles taught in the above references apply to transgene as well as knockout by homologous recombination. Thus, the phenotype resulting from targeted disruption of α1,3GT gene in different ovine strains or different farm animals would be expected and have been shown to be varied and unpredictable (See applicants' disclosure and references of *Denning et al, Dai et al, Lai et al,* and *Phelps et al*). *Logan and Sharma* (Clin Exp Pharmacol Physiol 1999 Dec;26:1020-25) teach "THE CHALLENGE IN THE DEVELOPMENT OF TRANSGENE IS NOT IN THIS PROCESS, BUT IN THE DESIGN OF THE CONSTRUCT THAT WILL ALLOW FOR THE EXPRESSION OF THE GENE OF INTEREST IN THE DESIRED CELL TYPE AT AN APPROPRIATE LEVEL". *Pearson* (Nature 2002;415:8-9) comments, "INDEED, CLEAR AND CONSISTENT PHENOTYPES NOW SEEM TO BE THE EXCEPTION RATHER THAN THE RULE" (left column, page 8). Clearly, long after the instant effective filing dates, animal transgenesis has not been considered as routine experimentation.

Applicants are further reminded that fetuses not surviving to term often reflect the real difficulty and challenge in animal cloning as taught by *Yanagimachi* (Mol Cell Endocrinol 2002;187:241-8) and *Wells et al* (Trends Biotechnol 2003:21:428-32) and cited foregoing. Thus, what is lacking in the specification and failed to achieve in the post-filing disclosure does not appear to be obtainable by routine experimentation.

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Q. Janice Li whose telephone number is 703-308-7942 (571-272-0730, after the Office relocation in January, 2004). The examiner can normally be reached on 9:30 am - 6 p.m., Monday through Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah J. Reynolds can be reached on 703-305-4051. The fax numbers for the organization where this application or proceeding is assigned are 703-872-9306.

Any inquiry of formal matters can be directed to the patent analyst, Dianiece Jacobs, whose telephone number is (703) 305-3388.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0196.

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